THE IMPACT OF BARIATRIC SURGERY ON INFLUENZA VACCINATION AND HOST METABOLISM

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ABSTRACT

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(Under the direction of Melinda A. Beck)

Bariatric surgery is the most effective method for weight loss in obese individuals. This is significant because obesity is associated with several co-morbidities, many of which have metabolic dysfunction as an underlying cause. One major obesity-associated co-morbidity is a compromised response to the influenza vaccine. Several questions remain unanswered with regard to bariatric surgery's impact on obesity associated co-morbidities. In our study, we analyzed the impact of bariatric surgery on vaccine induced serum antibody levels and circulating nutrient and metabolic hormone/peptide levels. Our findings suggest altered serum antibody levels are not the mechanism through which obesity compromises the response to influenza vaccination. Additionally, our findings highlight the remedial effect of bariatric surgery on host metabolism.

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TABLE OF CONTENTS

LIST OF TABLES AND FIGURES

LIST OF ABBREVIATIONS

HA	Hemagglutinin
NA	Neuraminidase
IgA	Immunoglobulin A
IgG	Immunoglobulin G
Th Cells	Th Helper Cells
CTLs	Cytotoxic T Lymphocytes
PBMC	Peripheral Blood Mononuclear Cell
HAI	Hemagglutinin Inhibition Assay
PBS	Phosphate Buffered Saline
RBC	Red Blood Cell
IOM	Institute of Medicine

CHAPTER 1: AIMS AND HYPOTHESIS

Obesity is characterized by a state of low grade chronic inflammation and has been shown to result in disturbed levels of circulating nutrients as well as metabolic hormones[14]. Obesity has also been recognized as an independent risk factor for increased mortality due to influenza virus infection[6]. Neidich et al. demonstrated that obese individuals have increased susceptibility to the influenza virus despite vaccination[16]. Our study will evaluate the impact of bariatric surgery on the immune response to influenza vaccine by measuring vaccine induced serum antibody levels before and after surgery. We will further quantify circulating metabolic hormones and nutrients to determine if bariatric surgery can re-establish a state of metabolic normalcy. We hypothesize that if obese individuals undergo bariatric surgery, their immune response to influenza vaccine will become more similar to the response seen in healthy individuals and the body will be returned to a state of metabolic normalcy.

Aim 1: ***Determine the effect of bariatric surgery on serum antibody levels against influenza.***

Obesity results in a compromised response to the influenza vaccine. We hypothesize that bariatric surgery should restore serum antibody levels to levels seen in healthy individuals.

Aim 2: ***Determine the effect of bariatric surgery on circulating nutrients and metabolic hormones/peptides.***

Obesity has been shown to result in disturbed levels of circulating nutrients and metabolic hormones/peptides. We hypothesize that bariatric surgery should return nutrient and hormone/peptide levels to those seen in healthy individuals.

Aim 3: ***Identify if any significant correlations between antibody levels and nutrient/metabolic hormone levels exist.***

Little is known of how obesity compromises the immune response to the influenza vaccine. Determining if any correlations exist between antibody levels against influenza and aspects of host metabolism may provide some insight. We hypothesize that serum antibody levels will be significantly correlated to aspects of host metabolism.

CHAPTER 2: INTRODUCTION

2.1 The Influenza Virus

Every year, between 5,000 and 56,000 Americans die as a result of the influenza infection[6]. Globally, the influenza virus causes between 250,000 and 500,000 deaths per year [6]. Influenza is a highly contagious respiratory tract infection that presents symptoms such as headaches, malaise, sore throat, congestion, body aches, and nausea or vomiting [6].

The influenza virus consists of segmented RNA encapsulated by a lipid envelope covered primarily by two viral peptides: hemagglutinin (HA) and neuraminidase (NA)[6]. HA and NA both recognize sialic acid, a diverse array of sugar units found on the carbohydrate side chains of cell-surface glycoproteins and glycolipids[4,27]. The initiation of influenza infection begins when HA recognizes and binds to sialic acids[4]. Once virus has replicated within a host cell, NA removes sialic acid from cellular glycoproteins and glycolipids, as well as from viral HA and NA[4]. The cleavage of sialic residues on the cell surface can facilitate the release of new virus[11]. Furthermore, the actions of NA prevent the virus from re-infecting the same cells and from aggregating with each other via HA-sialic acid interactions[4]. HA and NA proteins are typically the target of antibodies that block infection[4].

There are 4 strains of influenza: A, B, C, and D; A and B are the most common infection causing strains in humans[6]. To date, only influenza A viruses have been known to cause flu pandemics[1]. Influenza A viruses have various subtypes based on their hemagglutinin (HA) and neuraminidase (NA)[1]. There are 18 HA subtypes and 11 NA subtypes, meaning that a total of 198 different influenza subtypes are possible[1]. However, only 131 have been detected in nature[1].

Current subtypes that routinely circulate are A(H1N1) and A(H3N2); the currently circulating influenza A(H1N1) viruses are related to the 2009 flu pandemic[1].

Antigenic drift (mutations in virus genes that code for virus-surface proteins) in HA or NA which result from evolutionary pressures from host immunity allow for the virus to escape antibody detection generated against earlier influenzas strains. This can potentially cause influenza re-infection in the same individual[6].

2.2 The Adaptive Immune Response to Influenza

The adaptive immune response to influenza consists of humoral and cellular mediated immunity and is mediated by virus specific antibodies and T-cells.

2.21 Humoral Immunity

In response to influenza infection the body produces virus specific antibodies. The production of antibodies for HA and NA are particularly important because the presence of these antibodies correlates with protective immunity[11].

Immunoglobulin A (IgA) and immunoglobulin G (IgG) antibodies play a prominent role in protection against influenza[15]. Mucosal IgA antibodies are produced locally in mucosal tissues and provide a first line of defense at the site of infection[15]. The presence of serum IgA is indicative of a recent influenza virus infection[11]. IgG antibodies are important for systemic immunity and provide long lasting protection against a specific influenza strain[11]. HA and NA specific antibodies are of the IgG class antibodies[15].

HA specific antibodies bind to the trimeric globular head of HA and inhibit the attachment and entry of influenza into the host cell[11]. These antibodies can also assist the phagocytosis of viral particles by Fc receptor expressing cells[11]. Additionally, antibodies binding to HA expressed on infected cells can mediate antibody-dependent cell-mediated cytotoxicity,

which is a process in which the IgG antibody is recognized and acted upon by an effector cell[11,25]. HA antibodies therefore can neutralize the influenza virus and are a good correlate of protective immunity.

Antibodies specific to NA have protective potential[11]. NA specific antibodies inhibit the enzymatic activity of NA which facilitates the spread of virus via sialic acid cleavage. NA specific antibodies can also play a role in antibody-dependent cell mediated cytotoxicity to help clear virus infected cells.

2.22 Cellular Immunity

$CD4^+$ and $CD8^+$ T-cells are induced upon infection with influenza[11]. $CD4^+$ cells become active after recognizing MHC class II virus associated peptides on antigen presenting cells[11]. Although $CD4^+$ cells display cytolytic activity to a certain degree, their most important phenotype is that of T helper (Th) cells[11]. There are different subsets of Th cells based on their cytokine expression profiles. Th cells help coordinate the response of other immune cells through cell-cell interactions or by secreting cytokines after recognizing viral peptides bound to MHC class II molecules[23].

$CD8^+$ T cells primarily function in the form of cytotoxic T lymphocytes (CTLs) in response to viruses[11]. Upon influenza infection, these cells become activated in the lymphoid tissues and are recruited to the site of viral infection[11]. CTLs recognize virus infected cells and eliminate them via factors such as perforin and granzymes; perforins increase the permeability of the membranes of infected cells and granzymes induce apoptosis[11]. CTLs also release cytokines which improve antigen-presentation by inducing MHC expression, which further aids in detection of virally infected cells[11].

2.3 The Influenza Vaccine

Vaccination is the primary method used to prevent the onset of influenza[13]. Due to antigenic drifts, the influenza vaccine has to be reformulated on a yearly bases. Yearly vaccines are formulated by evaluating influenza strains which circulated in previous years[16]. The vaccine typically contains two influenza A strains and either one or two influenza B strains. The vaccine contains either inactivated virus or isolated viral HA and NA with HA being the primary immunogen in influenza vaccines[13]. Therefore vaccines are standardized to the quantity of HA[13].

Antibodies induced via influenza vaccination can be measured with a hemagglutinin inhibition assay (HAI). An HAI assay quantifies the protective antibodies blocking the ability of the virus to agglutinate red blood cells (RBCs). An HAI titer of 40 or greater represents the range at which 50% of individuals should be protected from infection[16]. Protection against influenza infection increases up to a titer of 160; beyond this titer, further protection capacity is minimal[16].

2.4 Obesity and Increased Susceptibility to Influenza

Obesity is classified as a state of low-grade chronic inflammation and includes altered levels of circulating nutrients, metabolic hormones, and excess adiposity[14]. Although a multitude of causes lead to obesity, it is primarily caused by prolonged positive energy balance[14]. There are several co-morbidities associated with obesity, one of which is immune dysfunction[14].

Various studies have shown altered immune cell function in obese individuals compared to healthy weight individuals. For example, it has been shown that circulating mononuclear cells in obese individuals display a pro-inflammatory state compared to healthy weight individuals[5]. Additionally, individuals with a mutation in the gene for leptin synthesis become morbidly obese and display a weakened immune response[3]. These, among other findings, suggest that obesity

can lead to an impaired immune system. One such consequence of the relationship between obesity and immune function is an increased risk of infection[14].

During the 2009 H1N1 influenza pandemic, obesity was recognized as an independent risk factor for increased morbidity and mortality due to the H1N1 infection[6]. Across the world data has shown that obese individuals were disproportionately represented among influenza related hospitalizations and deaths during the pandemic[14]. Kwong et. al demonstrated that during flu seasons in addition to the 2009 pandemic, obese individuals were at greater risk for respiratory hospitalizations[12].

In addition to increased susceptibility to infection, evidence suggests that obese individuals do not respond to vaccinations as effectively as healthy weight individuals[14]. Neidich et al. showed that vaccinated obese adults were twice as likely to develop influenza and influenza like symptoms compared to healthy weight adults[16].

Obesity has drastically risen over the past few decades. In the U.S. alone 37% of adults are obese[16]. Obese adults currently outnumber underweight adults, suggesting that the prevalence of obesity is a worldwide trend[6]. Given that obesity has been identified as an independent risk factor for influenza, this puts approximately 500 million obese individuals at risk for increased morbidity and mortality due to influenza worldwide[6].

2.5 Bariatric Surgery

The long term results yielded by traditional weight loss methods such as dieting, exercise, or medications are typically poor[9]. Bariatric surgery is recognized as the most effective treatment for weight loss[9]. Currently established procedures in the U.S. include sleeve gastrectomy and laparoscopic Roux-en-Y gastric bypass among others. These surgeries are typically very safe and have a mortality rate of 0.3%[9].

A sleeve gastrectomy, also known as a vertical sleeve gastrectomy or gastric sleeve procedure, involves the removal of the outer portion of the stomach leaving a small sleeve of stomach and the pylorus intact[8]. By reducing the size of the stomach, this purely restrictive procedure allows individuals to feel fuller after consuming fewer calories[8]. In a laproscopic Roux-en Y gastric bypass a large portion of the stomach is stapled off and the remaining upper portion is connected to a segment of the small intestine referred to as the Roux limb [21]. This not only reduces the amount of food an individual can consume but also reduces the absorption of nutrients from food by by-passing a portion of the intestine [21].

CHAPTER 3: METHODS

3.1 Study Design

Participants were recruited as part of a prospective observational study carried out at UNC Hospital's GI Surgery & Burn Specialty Clinic, located at 101 Manning Drive, Chapel Hill, NC 27599. All subjects gave written, informed consent as part of the UNC TraCS study on Visceral Inflammation and Immunometabolism in response to Bariatric Surgery (VIIBS), and the protocol was reviewed and approved by the University of North Carolina at Chapel Hill Institutional Review Board.

3.2 Participants

A total of 37 participants were enrolled in the study between March 2019 and January 2020. Recruitment criteria for the study were adults between the ages of 18 and 70 who consented to laparoscopic bariatric surgery. Exclusion criteria were individuals with immunocompromised systems, individuals taking immunosuppressing drugs, type 1 diabetics, and pregnant or breastfeeding women. At enrollment, informed consent, demographic characteristics and BMI were obtained. Blood samples from laparoscopic surgery candidates ages 30-65 years were obtained before and after surgery. Out of the 37 participants recruited, a sub-group of 14 participants was chosen for analysis. A total of 15 serum samples from individuals (ages 38-65 years) enrolled in a prior flu vaccine study were used as lean controls[16]. Lean controls were matched based on age and race.

3.3 Serum Isolation

Bariatric surgery patient blood samples were taken in vacutainer tubes and diluted in a 1:1 ratio with phosphate buffer saline (PBS)[10]. Samples were then layered on top of Lymphoprep in a

50 mL conical tube. Red blood cells, peripheral blood mononuclear cells, and serum were separated via density gradient centrifugation (1200g, 10 min)[7]. Serum was then collected with a Pasteur pipette and stored in a 2 mL cryogenic vial at -80°C. Lean control serum samples were obtained from a prior flu study[16].

3.4 Hemagglutination Inhibition (HAI) Assay

Using a Hemagglutination inhibition assay (HAI), antibodies against the influenza A(H1N1) 2009 virus in patient serum samples were quantified. The influenza A(H1N1) strain was used because it has been prevalent in many influenza vaccines over the past few years so it was likely that all participants would have been vaccinated against this strain. HAI titers were determined for all sera samples obtained from participants pre and post-bariatric surgery as well as for lean controls. HAI titers were determined in accordance with World Health Organization guidelines[29].

3.5 Luminex bi-plex cytokine kit

Bio-Plex multiplex immunoassays were used to quantify the presence of metabolic hormones and proteins in the sera of bariatric surgery patients before and after surgery and the sera of lean control patients. The Bio-Rad bio-plex pro human diabetes and adiponectin assay was utilized in accordance with the Bio-Rad protocol for the assay[20]. C-peptide, ghrelin, GIP, GLP-1, glucagon, insulin, leptin, PAI, resistin, visfatin, and adiponectin were quantified with the bio-plex immunoassay.

3.6 EPIC Database

Information regarding the study participants circulating nutrient levels at the time of their first and second blood draws were obtained from UNC healthcare's EPIC database. Glucose, iron,

ferritin, vitamins A, D, B1, B6, B12, A1c, AST, ALT, alkaline phosphatase, BUN, and creatine levels were obtained from EPIC.

3.7 Statistics

Sera samples were placed into sub-groups categorized as pre-bariatric surgery, post bariatric surgery, and lean control. Pre-bariatric surgery and post bariatric surgery samples were considered matched pairs. A Shapiro-Wilks normality test was used to assess the distribution of data for each variable being measured.

Variables were tested for significance between the three sub-groups. A Wilcoxon rank sum test or Mann Whitney test were used if the study population did not display normality for a variable. A Wilcoxon rank sum test was used for comparisons between matched pairs. A Mann-Whitney test was used for comparisons between unpaired values. A one-way ANOVA test was used for comparisons between variables that met the criteria for a Gaussian distribution; Tukey's multiple comparison test was used to further determine which sub-groups were statistically different. A p-value of a less than 0.05 was considered significant.

A Kendall Tau correlation test was used to correlate HAI titers to nutrient and metabolic hormone/peptide levels in serum. The Kendall Tau test was used over a Spearman test because of the repetitive nature of HAI titer measurements and because of the small sample size. A p-value of a less than 0.05 was considered significant.

All normality tests, tests of statistical difference and figures were run/created using the Graph Pad Prism version 8.41 software for macOS. All correlation tests were run using R-studio version 1.2.5033.

CHAPTER 4: RESULTS

4.1 Demographics

During the study year we enrolled 37 participants who underwent bariatric surgery at UNC Hospitals. Out of the 37 participants, 14 were selected for analysis of bariatric surgery's impact on the response to the influenza vaccine and the metabolic profile. Of the 14 participants that received the intervention, 29% were male and 71% were female. Approximately 79% of the participants were Caucasian and 21% were African American. The average age of participants who received the intervention was 44 years with a standard deviation of 11 years.

Table 1: Demographics of the Study Population

	Measure	Bariatric Surgery Participants	Lean Control Participants
Overall	Total Number	14	15
	Age	44±11	49±9
Sex	Male	4	4
	Female	10	11
Age	Caucasian	11	12
	African American	3	3

- Age is depicted as years ± standard deviation

4.2 Serum Antibody Titers Pre and Post-Bariatric Surgery

To investigate the impact of bariatric surgery on the immune response to influenza vaccination, we measured patient serum antibody titers against HA before and after bariatric surgery using HAI assays. Serum antibody titers were also measured for lean control participants. Two bariatric surgery patients had HAI titers which were determined to be outliers and were excluded from analysis. The population did not display a Gaussian distribution for HAI titers when tested with the Shapiro-Wilks normality test ($p=0.0001$) (Figure 1). Pre and post-bariatric surgery antibody levels were not significantly different from each other based on the Wilcoxon rank-sum test (Figure 2). When the population was stratified based on gender and race, pre and post-bariatric surgery antibody levels were still not significantly different from one another. On average, antibody titers decreased after surgery from an average titer of 80.00 to an average of 60.00. Antibody levels against HA in post-surgery patients became more similar to lean control individuals as evidenced by both mean and median (Table 2). HAI titers were not significantly different from lean controls both pre and post-surgery.

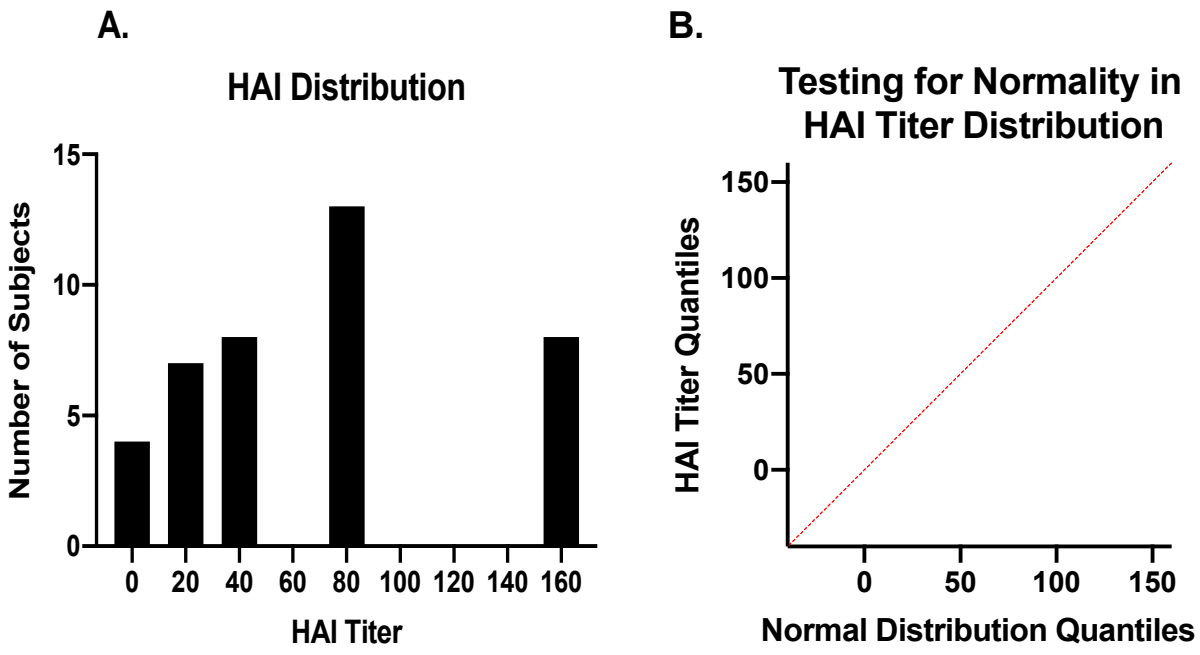

Figure 1: Distribution of HAI Titers in the Study Population

A: The distribution of the study participants HAI titers was not normal. B: Quantiles for the population HAI titers were not comprable to quantiles for a normal distribution.

Table 2. Descriptive Statistics of for HAI Titers Pre and Post-Surgery and for Lean Controls

Condition	Mean	Std. Dev.	Std. Error	Median
Pre-Surgery Titers	75.00	59.16	17.08	80.00
Post-Surgery Titers	65.00	53.34	15.40	60.00
Lean Control Titers	66.00	55.39	14.30	40.00

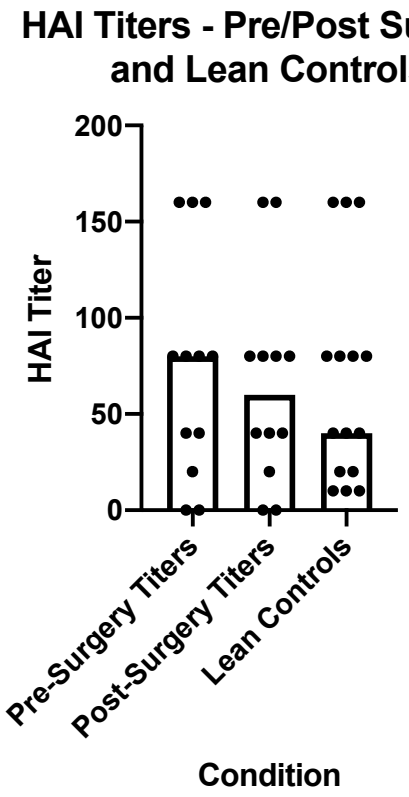

Figure 2: Comparisons between HAI Titers in the study sub-groups

Influenza A(H1N1) HAI titers for the pre-bariatric surgery, post bariatric surgery, and lean control participants. Pre and post-urgery titers were not statistically different. Pre and post-surgery titers were each not statistically different from lean control participant titers.

4.3 The Impact of Bariatric Surgery on Host Metabolism

In order to measure the impact of bariatric surgery on aspects of host metabolism, 25 nutrients and metabolic hormones/proteins were investigated (glucose, iron, ferritin, vitamins A, D, B1, B6, B12, A1c, AST, ALT, alkaline phosphatase, BUN, creatine, C-peptide, ghrelin, GIP, GLP-1, glucagon, insulin, leptin, PAI, resistin, visfatin, and adiponectin). Of these, sufficient data in regard to particpants iron, ferritin, vitamins, A, B1, and B6 were not available, and so these variabels were excluded from analysis. These variables were checked for statistical difference before and after surgery and for significance against lean controls. Variables that were statistically different before and after surgery are depicted in Table 3. The descriptive statistics for these variables are shown in Table 4. Figure 4 shows the mean with standard error or median level of visfatin, A1c, insulin, and resistin for each sub-group and depicts statistical significance between each group. These 4 variables saw a return to normalcy after surgery. The mean was used if the data was normally distributed, which was the case for visfatin.

Table 3: Distribution and Comparison to Lean Controls of Variables Statistically Different Pre vs. Post-Surgery

Variables Statistically different Pre vs Post-Surgery	Normal Distribution?	Statistically different Pre-Surgery vs. Lean Controls?	p-value	Statistically different Post-Surgery vs. Lean Controls?	p-value
Vitamin D (25 OH)	Yes	NA		NA	
Vitamin B12	Yes	NA		NA	
A1c	No	Yes	0.0263	No	0.5750
C-peptide	No	Yes	<0.0001	Yes	0.0358
Ghrelin	No	Yes	0.0034	Yes	0.0003
Glucagon	Yes	Yes	<0.0001	Yes	0.0020
Insulin	No	Yes	0.0003	No	0.0610
Leptin	No	Yes	<0.0001	Yes	0.0001
Resistin	No	Yes	0.0249	No	0.2604
Visfatin	Yes	Yes	0.0009	No	0.3963
Adiponectin	No	No	0.2115	No	0.4134

- UNC Healthcare's EPIC database lacked suficient data regarding the lean control participants Vitamin D and B-12 levels.

Table 4: Descriptive Statistics of Variables Statistically Different before and after Surgery

Variable	Sub-Group	Mean	Median
Vitamin D (25 OH) (ng/mL)	**Pre**	**22.87**	**22.20**
	Post	**33.73**	**33.60**
	Lean	**NA**	**NA**
Vitamin B12 (pg/mL)	Pre	456.0	452.0
	Post	703.1	732.0
	Lean	NA	NA
A1c	**Pre**	**5.964***	**5.6***
	Post	**5.28**	**5.3**
	Lean	**5.133**	**5.25**
C-peptide (pg/mL)	Pre	2296*	2376*
	Post	1572*	1556*
	Lean	1005	1014
Ghrelin (pg/mL)	**Pre**	**1391***	**1366***
	Post	**1256***	**1162***
	Lean	**2507**	**2542**
Glucagon (pg/mL)	Pre	3495*	3484*
	Post	3189*	3220*
	Lean	2923	2925
Inuslin (pg/mL)	**Pre**	**980.6***	**801.2***
	Post	**391.8**	**325.7**
	Lean	**259.8**	**221.8**
Leptin (pg/mL)	Pre	42756*	**46249***
	Post	20303*	**19361***
	Lean	3449	2756
Resistin (pg/mL)	**Pre**	**12758***	**10717***
	Post	**9703**	**8812**
	Lean	**7726**	**7215**
Visfatin (pg/mL)	Pre	16057*	16220*
	Post	14013	13907
	Lean	13089	13455
Adiponectin (pg/mL)	**Pre**	**8080283**	**8567250**
	Post	**9887750**	**9636200**
	Lean	**13512864**	**10746000**

- Variables are alternately bolded to aid in visually distinguishing between them.
- An asterisk (*) is indicative of statistical difference from the lean control

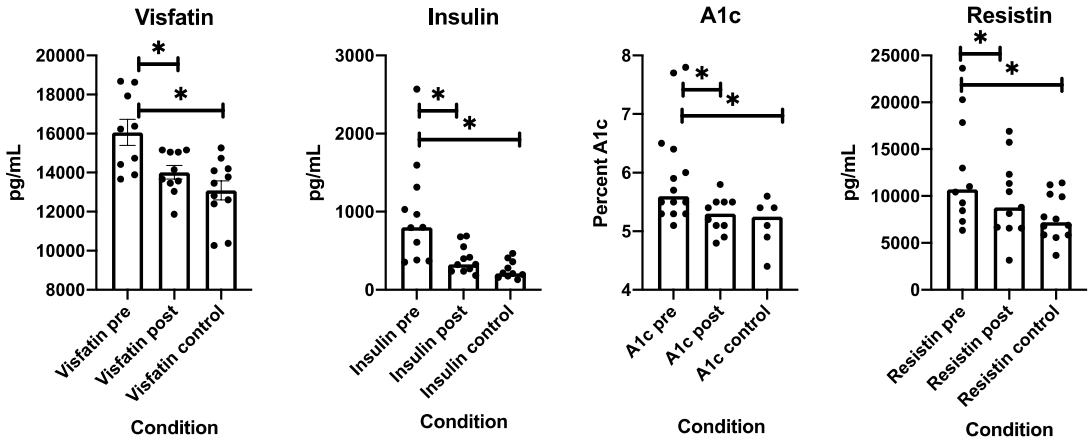

Figure 3: Comparisons between the 3 subgroups for Variables which saw a return to Normalcy

The 4 metabolic variables depicted by this figure each saw a return to normalcy after bariatric surgery. Data for each variable was statistically different pre vs. post-surgery. The pre-surgery data was shown to be statistically different from the control group. However, the post-surgery data for each variable was not statistically different from the control group. For visfatin, the mean with standard error is shown since the data for visfatin was normally distributed. The other three variables show the median levels since they did not display a Gaussian distribution.

4.4 HAI Titers as they relate to Cicrulating Nutrient and Hormone/Peptide levels.

HAI Titers were not significantly correlated with any of the nutrients or metabolic hormones/proteins measured. Based on the Kendall Tau test, correlations between HAI Titers and circulating nutrients and metabolic hormones/proteins had p-values of greater than 0.05.

CHAPTER 5: DISCUSSION

Obesity is a disease that affects 13% of the world's population and 37% of the U.S. population[16]. This disease, in addition to resulting in metabolic disturbances, has been identified as an independent risk factor for severity of influenza infection[6]. Furthermore, several studies have suggested that the response to influenza vaccination is impaired in obese individuals. This indicates that obesity not only plays a role in increasing the severity of this infection, but also plays a role in increasing susceptibility to the influenza virus.

Traditional weight loss methods such as dieting and exercise have been shown to return the body to a state of normalcy[9]. However, these methods have yielded poor weight loss results for obese individuals attempting to lose weight[9]. Bariatric surgery on the other hand has much higher rates of success when considering weight loss in obese individuals[9]. However, several questions remain unanswered with regards to how bariatric surgery can play a role in returning the body's immune system and metabolic profile to a state of normalcy.

5.1 Bariatric Surgery and Response to the Influenza Vaccine.

A key question being examined was whether or not bariatric surgery could improve an individual's response to the influenza vaccine. Our results showed that the median HAI titer of participants decreased from a titer of 80.00 before surgery to a titer of 60.00 after surgery. The post-surgery titer became more similar to the median HAI titer observed in lean control patients (40.00). However, our results indicated that there was no statistical difference between HAI titers before surgery and HAI titers after surgery ($p = 0.50$). Furthermore, our findings showed that neither pre-surgery nor post-surgery HAI titers were statistically different from the HAI titers of lean control participants ($p= 0.72$ and 0.99 respectively). When HAI titers were stratified based

on sex and race, HAI titers were still not statistically different pre vs. post-bariatric surgery or from lean control participants.

Our results would suggest that bariatric surgery has no significant impact on the body's production of serum antibodies against the influenza virus after vaccination. Furthermore, our results suggest that an obese individuals antibody production in response to vaccination is not significantly different from that of a healthy individual's. However, it is well established that obesity does lead to increased mortality and susceptibility to influenza virus. Therefore, we suggest that serum antibody levels induced by vaccination may not be not be an accurate measure of the body's ability to protect itself from the influenza virus, and other factors should be investigated instead (see future directions).

5.2 Changes in Metabolic Parameters as a Result of Surgery

Another key question this study aimed to answer was whether or not bariatric surgery could help re-establish a state of metabolic normalcy. A total of 11 metabolic variables were statistically different pre vs. post-surgery. Of the nutrients and metabolic hormones/peptides that were statistically different before and after surgery, data regarding four (vitamin D, vitamin B-12, glucagon, and visfatin) were normally distributed.

On average, vitamin D and B-12 levels both increased after surgery. There was no data regarding the lean participants vitamin D and B-12 levels, so comparisons to a healthy standard were made based on the Institute of Medicines (IOM) recommendations. The IOM recommends that adults need serum levels of at least 20 ng/mL for vitamin D and at least 250pg/mL for vitamin B-12 [17,18]. Both pre and post-surgery vitamin D and B-12 levels fell within a healthy range. Glucagon and visfatin levels both decreased after surgery and became more similar to levels seen in the control group. Glucagon levels were statistically different from the control

group both pre and post-surgery. Visfatin levels were significantly different from the control group pre-surgery but not post-surgery indicating that there was a return to normalcy.

Both vitamin D and B-12 levels are typically lower in obese individuals than healthy weight individuals[19,28]. Glucagon and visfatin levels are both typically elevated in obese individuals compared to healthy weight individuals[22,26]. The trends in these nutrient and hormone levels imply that bariatric surgery shifted their levels closer to normalcy, and in the case of visfatin re-established normalcy.

For the remaining seven variables, three saw a return to normalcy. The median A1c, insulin, and resistin levels decreased after surgery and became more similar to the healthy control median level. Furthermore, our results showed that there was a significant difference between pre-surgery levels and healthy control levels but no significant difference between the post-surgery levels and healthy control levels for these variables.

Obesity typically leads to the onset of type II diabetes, a disease characterized by insulin resistance. Therefore, obese individuals typically experience hyperinsulinemia and increased A1c levels[30]. Resistin levels are also typically elevated in obese individuals[2]. Therefore, our findings indicate that bariatric surgery helped return the levels of these metabolic variables to a state of normalcy. Our results showed that bariatric surgery did not have an impact on the number of type II diabetes diagnoses or on glucose levels, which is not in accordance with what our data states about insulin and A1c levels. However, it should be noted that all patients that were diagnosed with diabetes before surgery had their diagnoses removed 6 months after surgery.

Of the last four variables which did not see a return to normalcy, the median levels of three variables decreased after surgery. The median C-peptide and leptin levels decreased after

surgery and became more similar to the median level seen in healthy controls. However, both of these variables remained statistically different from the lean controls after surgery. The median Ghrelin level also decreased after surgery and was statistically different from the control group, however, it became more dissimilar from the lean control group than before surgery. Lastly, the median adiponectin level increased after surgery and became more similar to the median adiponectin level of the control group. Adiponectin levels were not statistically different form the levels seen in the control group pre or post-surgery.

Leptin levels are typically increased in obese individuals and adiponectin levels are decreased[26]. Our findings support that bariatric surgery helps shift leptin and adiponectin levels to levels seen in healthy individuals. However, post-surgery levels for Leptin were statistically different compared to lean control levels, implying that surgery is not an effective means of restoring leptin to normalcy or that more time after surgery needs to pass before significant change is seen. Adiponectin levels, although seeing a significant change after surgery, were not statistically different from the control group before or after surgery, implying that adiponectin was not significantly altered by obesity in our study population. Ghrelin levels were lower in the pre-surgery group compared to the control group, which was expected since ghrelin is typically downregulated in obesity[23]. However, after surgery levels continued to drop rather than increase. This may be a direct consequence of the surgery itself which removes the gastric fundus (the portion of the stomach that produces ghrelin) and would therefore result in lower amounts of ghrelin produced[31].

In summary, obesity led to trends in circulating nutrients and metabolic hormones/peptides that indicate a shift toward normality. It can only be said that 4 of these variables did indeed return to a state of normalcy based on statistical analysis. Of the 4 variables,

3 were markers of type II diabetes (visfatin, insulin, A1c). The return to normalcy of these variables in addition to the decrease in the number of type II diabetes diagnoses implies a remedial effect of bariatric surgery on this particular co-morbidity. Furthermore, the overall trend seen in the metabolic variables implies a remedial effect of bariatric surgery on the host metabolic profile.

5.3 Serum Antibody levels as they relate to the Metabolic Profile

The purpose in evaluating the relationships between vaccine induced serum antibody levels and metabolic parameters was to determine if the compromised response to the influenza vaccine seen in obese individuals could be linked to their altered metabolic profile. However, our findings indicated that no significant correlations were found between HAI titers and the nutrients or metabolic hormones/peptides measured. Correlations between variables were tested for individually in the three sub-groups (pre-surgery, post-surgery, and lean controls) and with the three sub-groups combined.

5.4 Limitations

One limitation of this study was the sample size. Our study included a total of 12 surgery patients, after accounting for outliers, and 15 participants in the control group for a total of 27 participants. The small sample size is likely the reason that the data for many variables was not normally distributed. Data that is not normally distributed is not generalizable to population. Therefore, while we can draw many conclusions for our study population based on our results, we cannot definitively not apply our conclusions to the wider population. Only data in regards to visfatin, insulin, A1c, and resistin were normally distributed, and therefore, our data in regards to these variables are applicable to the general population.

Another limitation of the study was that UNC Healthcare's EPIC database did not contain data regarding every participant in the study for certain variables. This made it impossible to draw a comparison of bariatric surgery participants vitamin D and B-12 levels to those of the lean controls. Additionally, some of the results of the bio-plex immunoassay were inconclusive, so data for some participants metabolic hormones and peptides levels was missing. These two limitations further decreased the sample size for data regarding certain variables and exacerbated the effects of an already small sample size, as mentioned in in the first limitation.

5.5 Future Directions

We recommend that this same study be carried out with a larger sample size to verify our findings. Due to our limitations, certain aspects of our study are not generalizable to the wider population and certain analyses may be skewed. We also recommend carrying out this for a longer duration of time in order to evaluate the effects of bariatric surgery beyond 4 months. This could provide further insights in regard to the procedure as it relates to influenza vaccination and host metabolism in case some of its effects require more time to surface. Furthermore, we recommend T-cell function be analyzed before and after bariatric surgery in order to determine if bariatric surgery has a significant impact on this aspect of immunity. T-cells from influenza vaccinated obese adults have been shown to be less activated when stimulated with vaccine strains of influenza[16]. Therefore, impaired T-cell function in obese individuals could be a reason for increased susceptibility to influenza. A more complete understanding of bariatric surgery's impact on T-cell function could help discern whether or not this surgery can help improve the immune response to influenza vaccine.

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